Department of Pesticide Regulation

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M E M O R A N D U M

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DATE: February 9, 2000

SUBJECT: BRAND NAME: DEF
ACTIVE INGREDIENT: S,S,S-Tributyl phosphorotrithioate (Tribuphos)
COMPANY NAME: Bayer Corporation
I.D. NUMBER: 181255
RECORD NUMBER (RN): 172610
DATA PACKAGE NUMBER (DPN): 272-108
EPA REGISTRATION NUMBER: --
TITLE: A dermal/intravenous crossover study to determine the dermal absorption of [^{14}C]-DEF 6 (S, S, S-Tributylphosphorotrithioate) in male rhesus monkeys

This memorandum contains brief description of methods used in a dermal absorption study of DEF 6 in five healthy male rhesus monkeys (*Macaca mulatta*). Bayer Corporation sponsored this study. As indicated in the submitted report, the study was conducted in accordance with the U.S. EPA Good Laboratory Practice standards (40 CFR, Part 160). The study was also conducted in accordance with the OECD and Japanese GLP standards. The following sections provide information on animal preparation, dose preparation, administration of the dose, analysis/recovery, results and recommendations.

Animal preparation

The ages of five male rhesus monkeys were approximately from 1.4 to 3.2 years old. Their body weights ranged from 3.1 to 3.6 kg at the time of the study assignment and 3.2 to 3.8 kg on day 15 after dose administration. These animals were housed individually in stainless-steel cages. The animal room was well ventilated (greater than 10 air changes per hour) with 100% fresh air (no air circulation). A 12-hour light/12-hour dark photoperiod was maintained. Room temperature ranged from 64 to 68 ^{o}F. Animals were provided with adequate food and water.

The study animals were acclimatized to their designated housing for 4 days before the first day of dosing. On the day prior to the dose administration, the animals were anesthetized with ketamine HCl. Then a sufficient area of the back was shaved for dose administration. The entire shaved area was cleansed with water and patted dry. On the morning of dose administration, food was withheld. Animals were anesthetized and catheterized for urine and blood collection.

The animals were placed in primate restraint chairs. The dosing site was accessible from the front of the chair and the arms and legs were secured. A Duoderm® patch was placed around the dosing site to expose an area of 24 cm^2 (4 cm x 6 cm).

Dose preparation

Research and Development Department of Bayer Corporation, Agriculture Division, Kansas prepared the ready-to-use dosing solution. The final dosing solution was the suspension of DEF-1-^{14}C- and DEF 6 formulation blank in distilled water. The concentration of the dosing solution was 7.35 µCi of DEF/100 µL. Results of the analysis indicated that the radioactivity was still DEF 1 and the radiochemical purity of the solution was 100%, indicating that the ^{14}C-DEF 6 was stable. The material was packaged and shipped to Sierra Biomedical, Incorporated (SBi) in a conical-bottomed vial containing a magnetic stir-bar, and closed with a screw-cap containing a rubber/Teflon laminated disc.

Administration of the dose

SBi discovered that the radioactivity of dosing solution was lower than 7.35 µCi of DEF 6/100 µL as indicated on the shipping label. SBi conducted a series of test and analysis. But, the radioactivity was still lower than expected. Analysis of the rubber/Teflon laminated disc and Teflon magnetic stir-bar of the returned sample vial indicated that these materials probably adsorbed ^{14}C-DEF, resulting in lower counts of radioactivity. To resolve this problem, SBi used the average of the radioactivity in the 100 µL samples taken from the dosing vial prior to and after dosing to animals to calculate the administered dose to each animal. In order to accomplish this procedure, the solution in the dosing vial was stirred continuously by a magnetic stirrer when samples were taken for analysis and for dosing to animals. Each animal was dosed with 100 µL of dosing solution by using a calibrated glass micropipet. The mean dose of [^{14}C]-DEF 6 administered to the animals was 83.3 µg/24 cm^2 or 3.5 µg/cm^2. Only dermal administration of the dose was performed in those monkeys. Intravenous injection was unnecessary because the recovery of radioactivity was greater than 90%.

The application site was covered with aluminum dome and secured. After an exposure period of 8 hours, the animals were anesthetized with ketamine HCl and removed from the restraint chairs. The protective dome and the Duoderm® patch were removed. The surface of the test site was washed with a series of 16 soap/water soaked cotton-tipped swabs (1% Ivory liquid soap diluted with tap water) and 2 dry swabs. The urine catheter and blood collection catheter were removed and discarded. Then the animals were returned to the housing cages. Tape stripping was performed 48 hours after dosing. An area of approximately 4 cm x 1.5 cm was tape stripped 16 times using cellophane tape. The purpose of tape stripping is to determine if the dosed radioactivity was trapped on the skin and was slowly absorbed and excreted.

All necessary samples were collected for analysis according to the study protocol. Urine and feces collection intervals were 0-4, 4-8, 8-12, 12-24, 24-48, 48-72, 72-96, and 96-120 hours, and approximate 24-hour intervals after the 120-hour time point. The animals were released from the

study when the recovered radioactivity was greater than 80% (actual average recovery was 105.3%), and skin and urine radioactivity levels were at or below twice the background.

Analysis/recovery

A Beckman liquid scintillation spectrophotometer was used to analyze radioactivity in various samples obtained from the study. Prior to analysis, these samples were properly prepared (e.g., combusted, extracted) depending on the types of samples. Insta-Gel was added to samples before analysis.

Total recoveries of radioactivity from individual animals ranged from 100.6 to 109.0% with a mean of 105.3%. The mean percent recoveries in various samples were: 6.24 (urine), 0.72 (feces), 0.48 (biscuits), 1.25 (dermal dome), 2.73 (Duoderm®), 93.8 (dermal swabs), 0.08 (tape strips).

Results

Results of radioactivity analysis in samples are shown as percent of administered dose (Table 1).

Table 1. Total radioactivity expressed as percent of administered dose from five monkeys.

	Dermal group samples							
Monkey No.	Urine	Feces	Biscuits	Tape strips	Dermal dome	Swabs	Duoderm®	Total recovery
R10734M	9.19	0.62	0.58	0.07	0.95	87.01	2.19	100.6
R11070M	8.47	0.68	0.59	0.08	0.67	94.15	4.33	109.0
R10729M	3.57	0.87	0.37	0.07	0.66	99.84	0.68	106.1
R10745M	4.93	0.55	0.50	0.06	0.96	96.71	1.91	105.6
R10712M	5.03	0.88	0.38	0.10	3.03	91.38	4.52	105.3
Mean	6.24	0.72	0.48	0.08	1.25	93.82	2.73	105.3
± SD	2.45	0.15	0.11	0.02	1.00	4.93	1.65	3.01

Dermal absorption (% dose in urine + feces + biscuits) = 7.44 ± 2.47%
Adjusted dermal absorption based on the mean recovery of 105.3% = 7.1 ± 2.3%

An excretion profile was analyzed by employing the exponential saturation model (Thongsinthusak *et al*., 1999). This exercise used cumulative dose recovered in urine, feces and biscuits (Table 2). The excretion profile is deemed necessary because the manner in which the administered dose was determined in the study. This is because the actual concentration of DEF 6 in the dosing solution was inconsistent with that shown on the shipping label. The excretion profile will assist in determining if there is an unusual pattern of excretion of DEF in the study animals.

Table 2. Mean cumulative recovery of [^{14}C]-DEF 6 in urine, feces and biscuits of five male rhesus monkeys.

	Cumulative excretion (% dose)			
Time interval (hour)	Urine	Feces	Biscuits	Total
0-4	0.08	0.01	0.0	0.09
4-8	0.59	0.03	0.0	0.62
8-12	1.16	0.03	0.01	1.20
12-24	1.39	0.17	0.09	1.65
24-48	4.04	0.34	0.19	4.57
48-72	5.09	0.46	0.41	5.96
72-96	5.52	0.58	0.46	6.56
96-120	5.84	0.74	0.48	7.06

Results of analysis showed that the maximum excretion of the administered dose was 8.54% (Figure 1). When this value was adjusted for the average recovery of 105.3%, the maximum excretion of the dose was 8.1%. Since the administered dose excreted and collected only in excreta and biscuits, the extrapolated dermal absorption was determined to be 8.1%. The graph in Figure 1 shows that the excretion of DEF 6 follows normal excretion patterns observed in other studies (Thongsinthusak *et al*, 1999). However, the excretion values found in samples collected at and after 96 hours should be slightly higher than the observed values.

Recommendations

1. This dermal absorption study is acceptable for use in the determination of dermal absorption of DEF. Even though there was some error in the preparation of the dosing solution, the actual dose was determined from samples taken at the time of dose administration.

2. An observed dermal absorption value of 7.1% is recommended for use in the calculation of absorbed dose.

Reference

Thongsinthusak, T., Ross, J. H., Saiz, G. S., and Krieger, R. I. 1999. Estimation of dermal absorption using the exponential saturation model. *Regul. Toxicol. Pharmacol.* 29:37-43.

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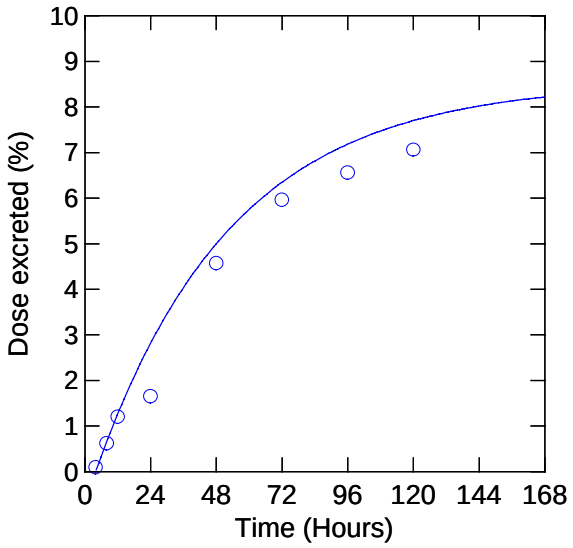
(TCW/Dermal/HSM-00001)

Figure 1. Excretion profile of [^{14}C]-DEF 6 for five male rhesus monkeys after dermal administration at 3.5 µg/cm^2.



Statistics:

SYSTAT Rectangular file D:\DATA\TCSYS\Def04.sys,
created Fri Jan 21, 2000 at 09:11:49, contains variables:

Iteration

No.	Loss	MAX	RATE	LAG
0	.635801D+01	.101000D+02	.102000D-01	.103000D+02
1	.492795D+01	.700191D+01	.162287D-01	.239002D+01
2	.705549D+00	.854827D+01	.159468D-01	.431641D+01
3	.694699D+00	.854084D+01	.160362D-01	.397795D+01
4	.694698D+00	.854349D+01	.160260D-01	.397595D+01
5	.694698D+00	.854320D+01	.160272D-01	.397626D+01
6	.694698D+00	.854323D+01	.160270D-01	.397623D+01

Dependent variable is RECOV

Source	Sum-of-Squares	df	Mean-Square
Regression	153.14	3	51.05
Residual	0.69	5	0.14
Total	153.84	8	
Mean corrected	57.86	7	

Raw R-square (1-Residual/Total) = 1.00

Mean corrected R-square (1-Residual/Corrected) = 0.99

R(observed vs predicted) square = 0.99

Wald Confidence Interval

Parameter	Estimate	A.S.E.	Param/ASE	Lower < 95%>	Upper
MAX	8.54	1.01	8.50	5.96	11.13
RATE	0.02	0.00	3.93	0.01	0.03
LAG	3.98	1.88	2.11	-0.86	8.82